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EXAMINER	
SWOPE, SHERIDAN	

ART UNIT	PAPER NUMBER
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/573,821	OKUNO ET AL.	
	Examiner	Art Unit	
	SHERIDAN SWOPE	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 March 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *49,51-56,59-62,67 and 68* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *49, 51-56, 59-62, 67, and 68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *1107*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' request for continued examination of March 15, 2011, in response to the action mailed October 1, 2010, is acknowledged. It is acknowledged that Claims 36-48, 50, 57, 58, and 63-66 have been cancelled, and Claims 49, 51, 55, 59-62, and 67 have been amended. Claims 49, 51-56, 59-62, 67, and 68 are pending. The invention under prosecution is drawn to a method for cleavage using an E. coli OmpT protease or a variant thereof having a substitution at Asp^{97}. Claims 49, 51-56, 59-62, 67, and 68 are herein reconsidered.

Advisory Action

Regarding the Advisory Action of February 16, 2011, Applicants make the following comments. The Office alleges that "Amendment of Claim 49 and 51 requires additional search and consideration." Applicants disagree. Claim 49 is amended to incorporate elements from claims 50 and 63-65 (now canceled). No additional search and/or consideration should be required, because claims 50 and 63-65 previously have been searched. Claim 51 is amended to change passive voice to active voice. Applicants submit that no additional search and/or consideration should be required for such an amendment.

These comments are not persuasive. MPEP 803.02 states:

> "...the Markush-type claim will be examined fully with respect to the elected species and further to the extent necessary to determine patentability." (Examiner's emphasis)

Thus, the Office is not required to search every species of a Markush claim. Rejection of a single species is sufficient to reject the claim. It is noted that, based on the amendments to Claim 49, additional searching of the databases EAST, STN, and GOOGLESCHOLAR was required (enclosed).

Special Status

Applicants state that "On and after March 28, 2011, the application ... will have a 'special' status under M.P.E.P. § 707.02 and should be processed accordingly." It is acknowledged that the instant Application was filed on March 28, 2006 and, thus, has "special" status under M.P.E.P. § 707.02. Therefore, the application has been carefully studied by the supervisory patent examiner.

Priority

For the instant claims, priority is granted to PCT/JP04/14704, filed September 29, 2004, which disclosed the recited invention. The examiner cannot consider whether JP 2003-342183, filed September 20, 2003, disclosed the recited invention because an English translation thereof has not been made available by applicants.

Claim Rejections - 35 USC § 112-Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Rejection of Claims 49, 51-56, 59-62, 67, and 68 under 35 U.S.C. 112, second paragraph, as being indefinite is maintained for the following reasons and those stated in the prior actions.

For Claim 51, the phrase "wherein the amino acid sequence from a P 10 position to a P3 position consists of a single basic amino acid or two or three consecutive basic amino acids" renders the claim indefinite. The amino acid sequence from a P10 position to a P3 consists of eight amino acids and, thus, cannot "consists of a single basic amino acid or two or three consecutive basic amino acids". The skilled artisan would not know the metes and bounds of the recited invention. Claims 52 and 53, as dependent from Claim 51, are indefinite for the same reason. For purposes of examination, it is assumed that "wherein the amino acid sequence from a P 10 position to a P3 position consists of a single basic amino acid or two or three consecutive basic amino acids" means "wherein the amino acid

sequence from a P10 position to a P3 position comprises only a single basic amino acid or only two or three consecutive basic amino acids".

Claims 51-53 and 55 are rendered indefinite for improper antecedent usage as follows.

Rejection of Claim 55 because the phrases "a P10 position" and "a P3 position" should be corrected to "the P10 position" and "the P3 position" is maintained.

For Claim 51, as amended, the phrases "a P10 position" and "a P3 position" should be corrected to "the P10 position" and "the P3 position". Claims 52 and 53, as dependent from Claim 51, are indefinite for the same reason.

Relevant to these rejections of Claims 51-53 and 55 for improper antecedent usage, Applicants argue that claims 51 and 55 correctly recite "a P 10 position" and "a P3 position" when these positions are first recited. This argument is not found to be persuasive because it is unclear whether the phrases "a P10 position" and "a P3 position" refer to (i) the positions relevant to positions P1 and P1', as recited in Claims 49 and 54 or (ii) positions not relevant to positions P1 and P1', as recited in Claims 49 and 54. The skilled artisan would not know the metes and bounds of the recited invention.

Any subsequent rejection based, on clarification of the above phrases and terms, will not be considered a new ground for rejection.

Claim Rejections - 35 USC § 112-First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Written Description

Rejection of Claims 49, 51-56, 59-62, 67, and 68 under U.S.C. 112, first paragraph/written description, for reasons set forth in the prior action, is maintained.

In support of their request that said rejection be withdrawn, Applicants provide the following arguments. These arguments are not found to be persuasive for the reasons following each argument.

(A) Whether generic claims to biological subject matter comply with the written description requirement is determined by an analysis of the Capon factors.

(A) Reply: It is acknowledged that Capon v Eshhar is a case relevant to the written description requirement.

(B) The Office's assertion as to inadequacy of working examples ("functional permutations") is unsupported. The Specification provides multiple working examples, wherein OmpT cleaves numerous motifs within the scope of the claims. See, e.g., Specification, Examples 2, 4, 6, 8, 10, and 17-18.

(B) Reply: It is acknowledged that exemplification of each and every embodiment is not required. Nonetheless, as explained in the prior action (p3-4), based on Applicants' arguments, it has been assumed that "OmpT protease" means any E. coli protein having any structure and having any activity of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1). And, that the instant claims encompass any variant of said E. coli proteins having any structure comprising a substitution of a residue corresponding to Asp97 of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1). The specification fails to disclose using any variant of any protein other than the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1).

Moreover, the instant claims encompass using any said variant to cleave an essentially unlimited number of substrates; see the action of October 1, 2010, p6 ¶1. The specification fails to describe the encompassed scope of cleavage methods for the reasons explained in the prior action, in part, as follows.

(i) The specification discloses the substrate can be up to 15AAs long (Claim 1; P10-P5'), which represents 10^{17} of possible substrates.

(ii) The substrate specificity is unpredictable for the following reasons.

The specification states:

"Sugimura et al. have examined the substrate specificity of OmpT protease and have reported that the enzyme specifically cleaves the central peptide bonds between the basic amino acid pairs of arginine-arginine, lysine-lysine, arginine-lysine and lysine-arginine (Sugimura, K. and Nishihara, T. J. Bacteriol. 170: 5625-5632, 1988)." [0003]

"However, the enzyme does not cleave all basic amino acid pairs, as it is highly specific. For example, human γ-interferon contains 10 basic amino acid pairs, but only two of them are cleaved (Sugimura, K. and Higashi, N.J. Bacteriol. 170: 3650-3654, 1988). This is attributed to the influence of the three-dimensional structure of the human γ-interferon substrate and to the amino acid sequences of sites thought to be recognized by the enzyme which are adjacent to basic amino acid pairs." [0004]

"OmpT protease cleavage sites have been discovered with amino acid sequences other than basic amino acid pairs, and Dekker et al., using substrates with amino acid substitutions introduced into an OmpT protease substrate comprising the amino acid sequence Ala-Arg-Arg-Ala (P2-P1↓P1'-P2'), have reported that OmpT protease exhibits high specificity for the basic amino acids arginine and lysine as the amino acid at the P1 position of the cleavage site, but is less stringent in regard to the amino acid at the P1' position (Dekker, N. et al. Biochemistry 40: 1694-1701, 2001)." [0008]

For the OmpT protease of Sugimura et al, 1988a, only 8AAs, P4-P4', have been examined in the art regarding substrate specificity. McCarter et al, 2004, teaches that published reports regarding the specificity of OmpT protease specificity are inconsistent (see p5923, col2, ¶1&2-p5924,¶1). Thus, the public cannot deduce the OmpT specificity at P10-P5' (original Claim 1) or assume that the variants have the same specificity; the substrate specificity for the encompassed scope is unpredictable.

In addition, as acknowledged by Applicants, "the present application employs a different substrate than the one in Kramer", which supports the Office's position that cleavage of Arg↓Arg, and other P1↓P1'motifs, by OmpT variants is dependent on residues outside of the P1 and P1' positions and remained unpredictable.

Regarding the substrates cleaved by the recited variants:

(i) For the single variant having D97L, the specification teaches: cleavage of the site RAR↓SSYSME (Fig11-12), and minimal cleavage of sites LRLYRS↓[A/F/S/C/Y]HHGS (Exmp13 &Table 1), ie only 6 substrates. These working examples are nothing like the encompassed scope of possible substrates (10^{17}).

(i) For the single variant having D97M, the specification teaches: cleavage of AAR↓RR↓AR↓FVPIF and DARRR↓AR↓FVPIF (Fig5,15) and minimal cleavage of LRLYR↓[A/V/F/S/C/Y]HHGS (Exmpl3;Table 1), ie only 8 substrates. These working examples are nothing like the encompassed scope of possible substrates (10^{17}).

(iii) For the single variant having D97H, the specification teaches: cleavage of AARRR↓AR↓CGNLS (Fig 11-12) and minimal cleavage of

LRLYR↓[A/V/I/F/M/S/T/C/N/K/R]HHGS ('Exmpl3;Table 1), ie only 13 substrates. These working examples are nothing like the encompassed scope of possible substrates (10^{17}).

Thus, the full scope of variant proteases and substrates thereof is not described such that the skilled artisan would recognize possession.

For these reasons and those explained in the prior action, Claims 49, 51-56, 59-62, 67, and 68 are rejected under 35 U.S.C. 112, first paragraph/written description.

Claim Rejections - 35 USC § 102

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 49 and 51-55 rejected under 35 U.S.C. 102(a) as being anticipated by Okuno et al, 2004 (IDS). Okuno et al teaches a process for cleaving fusion proteins using variants of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1) having substitutions at Asp^{97}. The recombinant variant having Leu^{97}, cleaves fusion proteins having an R at position P1, an S or A at position P1', and an R at position P4 (Table 1). The recombinant variant having Met^{97}, cleaves fusion proteins having an R at position P1, an F, A, S, C, or Y at position P1', and an R at position P4 (Table 1). The recombinant variant having His^{97}, cleaves fusion proteins having an R at position P1, an A, V, I, M, S, T, C, or N at position P1', and an R at position P4 (Table 1). Therefore, Claims 49 and 51-55 rejected under 35 U.S.C. 102(a) as being anticipated by Okuno et al, 2004.

Claims 59-61, 67, and 68 are rejected under 35 U.S.C. 102(a) as being anticipated by Okuno et al, 2004. Okuno et al teaches a process for cleaving fusion proteins using variants of the E. coli OmpT protease of Sugimura et al, 1988a (AAA24430.1) having a substitution of Asp^{97}. The variant having Met^{97}, cleaves fusion proteins having an R at position P1, an F at position P1', and three consecutive Rs at positions P5-P3, wherein the target protein, motilin, is released upon cleavage (Fig 4).

Therefore, Claims 59-61, 67, and 68 are rejected under 35 U.S.C. 102(a) as being anticipated by Okuno et al, 2004.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 56 is rejected under 35 U.S.C. 103(a) as being unpatentable over Okuno et al, 2004 in view of Lejal et al, 2000. The teachings of Okuno et al are described above. Okuno et al does not teach a method of cleaving a polypeptide by coexpressing the polypeptide with the OmpT protease variant. However, cleaving a polypeptide by coexpressing the polypeptide with a protease was well known in the art (Lejal et al; Fig 5-7). It would have been obvious to a person of ordinary skill in the art to combine the teachings of Okuno et al and Lejal et al to make a method for cleaving fusion proteins using variants of OmpT protease, as taught by Okuno et al, wherein the fusion protein and variant are co-expressed in host cells. Motivation to do so is provided by the desire to cleave the fusion protein without purification. The expectation of success is high, as all steps and reagents were known in the art. Therefore, Claim 56 is rejected under 35 U.S.C. 103(a) as being unpatentable over Okuno et al, 2004 in view of Lejal et al, 2000.

Claims 49 and 51-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. As described in the prior action, the combination of Okuno et al, 2002a, Dekker et al, and Kramer et al renders obvious using the $Asp^{97}Ala$ OmpT protease variant of Kramer et al to cleave one

or more substrates wherein the P1' position is not Arg or Lys. Said combination does not teach cleavage of one or more of said substrates with an OmpT protease variant having a substitution at Asp^{97} with an amino acid other than Ala. Nonetheless, it would have been obvious to a person of ordinary skill in the art to make the OmpT protease AAA24430.1 variants having a substitution at Asp^{97} with each of the naturally occurring amino acids, including **Leu** and **Met,** and use said variants to cleave the fusion proteins of Okuno et al, 2002a (Tables 1&2) and the peptides of Dekker et al (Table 2), including those having a P1' of Ala or Phe. It is noted that the fusion proteins of Okuno et al, 2002a have the basic residue Arg at P4 (Tables 1&2). Motivation to do so is provided by the desire to examine the cleavage motif requirements of said variants by using the sequences of Okuno et al, and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that, more likely than not the OmpT protease variants having a substitution at Asp^{97} with **Leu** or **Met** would cleave a substrate comprising a P1' that is S, A, F, C, Y, or V. This expectation of success is based on the fact that (i) Kramer et al teaches that the residue at position 97 of OmpT protease determines substrate specificity in regards to position P1' (Fig 4), (ii) neutral/hydrophobic amino acids are more likely to associate with each other (Metzler), and (iii) substrate binding and cleavage is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 49 and 51-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001.

Applicants' relevant arguments

As acknowledged by Applicants' (section 13.1) the prior rejection that is relevant to the rejection above is "(8) claims 50, 63, and 64 over the combination of Okuno 2002a, Dekker, and Kramer, in view of Metzler". Applicants did not make any comments regarding this specific rejection

because Claims 50 and 63-64 are cancelled. Claim 49 now encompasses the subject matter of prior Claims 50, 63, and 64. Applicants make the following arguments (sections 13.2&13.5) regarding Claim 49, as amended, which are relevant to the rejection of Claims 49 and 51-53 above.

(A) To render a claim obvious, both the suggestion of the claimed invention and the expectation of success must be in the prior art, not from the disclosure of the claimed invention. Additionally, obviousness requires a suggestion of all limitations in a claim. The Office must also establish that one of ordinary skill in the art would have had a reasonable expectation of success to practice the claimed invention.

(A) Reply: KSR International vs Teleflex Inc. (Federal Register/ Vol. 72, No. 1995, October 10, 2007) takes precedent in the Office's current determination of obviousness under §103(a). Therein, rationales supporting an obviousness rejection are (72 Fed. Reg. 57526; esp pg 57529):

(a) combining prior art elements according to known methods to yield predictable results,
(b) simple substitution of one known element for another to obtain predictable results,
(c) use of a known technique to improve similar devices (methods or products) in the same way,
(d) applying a known method to a known product or method ready for improvement to yield a predictable result,
(e) "obvious to try" –choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success,
(f) known work in one filed of endeavor may prompt variations for use in the same or a different field, if the variants would have been predictable, and
(g) some teaching, suggestion, or motivation in the prior art to lead the skilled artisan to modify or combine prior art teachings.

In the instant case, rationales above for supporting the obviousness rejection are (a) combining the prior art references will, more likely than not, lead to the predicated result of using the OmpT protease variants having a substitution at Asp^{97} with **Leu** or **Met** to cleave a substrate comprising a P1' that is S, A, F, C, Y, or V, (b) simple substitution of one known element for another to obtain said predictable result, (d) applying a known method to a known product for improvement to yield a

predictable result, and (e) "obvious to try" –choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. The reasons for a reasonable expectation of success are explained in the above rejection.

(B) Kramer does not teach any of the recited E. coli OmpT protease 97th amino acid variants (i.e., D97L, D97M, and D97H). Thus, the combination of Sugimura 1988a, Okuno 2002a, Okuno 2002b, Dekker, and Kramer fails to teach at least the recited E. coli OmpT protease 97th amino acid variants. Without all claim elements taught, there can be no expectation that the presently claimed methods would have worked predictably.

(B) Reply: It is acknowledged that Kramer does not teach any of the recited E. coli OmpT protease 97th amino acid variants (i.e., D97L, D97M, and D97H). Nonetheless, it would have been obvious to the skilled artisan to substitute the D97A mutation of Kramer with other mutations at said position. Such a strategy is common and well-known in the art; see, for example, Scheer et al, 1997.

(C) Furthermore, the OmpT D97A variant of Kramer cleaved the Abz-Ala-Arg↓Arg-Ala-Dap(dnp)-Gly substrate with only 6% efficiency, relative to the wild-type OmpT. The Office has not explained why the skilled artisan, knowing that an OmpT D97A variant shows only 6% of the wild-type activity, would have been directed to make additional variants (e.g., OmpT D97L, D97M, and/or D97H) to cleave any of the substrates taught in Sugimura 1988a, Okuno 2002a, Okuno 2002b, and Dekker.

(C) Reply: As explained in the prior action, Kramer et al teaches that the amino acid in position 97 of OmpT protease determines the substrate specificity regarding position P1' (Fig 4). Thus, the skilled artisan would have been motivated to make the variants of OmpT protease having each of the naturally occurring amino acids at position 97 and test the substrate specificity for position P1'. Both Okuno et al, 2002a and Dekker et al teach genera of substrates having every one of the 20

naturally occurring amino acids at position P1'. Thus, the skilled artisan would have been motivated to test the position 97 variants of OmpT protease using the substrates of Okuno et al, 2002a and Dekker et al. Moreover, the art provides guidance as to which position 97 variants of OmpT are likely to cleave which P1' substrates (Metzler, 2001 and Wolf et al, 1995).

(D) Applicants submit that Kramer actually teaches away from making 97th amino acid variants, because Kramer's only attempt to make such a variant produces poor results. Additionally, there is no evidence on the record that a skilled artisan, from all the taught substrates, would have been directed to select only those presently recited, let alone that the cleavage would have been worked predictably.

(D) Reply: Kramer found poor results with only a single variant, OmpT D97A, using only a single wild-type OmpT substrate. See Reply (C), above.

(E) Finally, the Office's assertion as to substrate binding and cleavage coordination is unsupported. According to Fig.4 of Kramer, multiple Asp residues (e.g., Asp83, Asp85, Asp208, and Asp210) are also involved in the formation of OmpT protease's active site and thus required for substrate binding and cleavage coordination. The Office fails to provide a rationale why a skilled artisan would have been directed to instead substitute the Asp residue at the 97th position among other Asp residues.

(E) Reply: It is acknowledged that Asp83, Asp85, Asp208, Asp210 are also involved in cleavage activity. However, only Asp210 has been taught by Kramer et al as being involved in substrate specificity, i.e. for position P1 (p428 ¶3). Thus, for making OmpT variants having altered substrate specificity, skilled artisan would substitute positions Asp210 and Asp97 with each of the naturally occurring amino acids and test for P1 and P1' substrate specificity, respectively. As explained above, for the Asp97 variants, it would be obvious to test the substrates of Okuno et al,

2002a and Dekker et al.

(F) Even if the Office's reliance on and interpretation of Metzler were proper (Applicants do not necessarily agree), the claimed methods offer unexpected results. Applicants respectfully direct the Office to Table 1, at page 46 of the Specification. The claimed OmpT D97L variant (Asp97 substituted by leucine) cleaves PRS (serine at the P'1 position) at a higher efficiency than PRA (alanine at the P'1 position). Similarly, Table 1 of the Specification indicates that the claimed OmpT D97M variant (Asp97 substituted by methionine) cleaves PRS at a higher efficiency than PRA. Both of these observations are contrary to the Office's interpretation of Metzler, which would have concluded that OmpT D97L or OmpT D97M would have a higher cleavage activity toward a substrate having I, F, V, A, Y, M, W, or L at the P'1 position ("neutral/hydrophobic amino acids are more likely to associate with each other").

(F) Reply: It is acknowledged that the D97L variant cleaves PRS at a 22% higher efficiency than PRA. However, the D97L variant cleaves both PRS and PRA, as would be predicted by Metzler. It is acknowledged that the D97M variant cleaves PRS at a 15% higher efficiency than PRA. However, the D97M variant cleaves both PRS and PRA, as would be predicted by Metzler. It would not be unexpected that there would be modest variability between substrates have different, but common, amino acids in the P1' position.

(G) Based on Fig. 4 of Kramer, a skilled artisan may have expected that an OmpT variant having a basic amino acid at the 97th position would have had a high cleavage specificity toward a substrate having an acidic amino acid at the P'1 position. However, the present inventors have found that OmpT D97H variant (histidine is a typical basic amino acid according to Metzler) hardly cleaved substrates having an acidic amino acid at the P'1 position. See Example 13 and page 47, ¶1. Instead, OmpT D97H effectively cleaves substrates having a neutral amino acid (e.g., alanine and valine) at the

P'1 position. As shown in Table 1 of the Specification, OmpT D97H (1) cleaves PRA at an efficiency of 8.4% and (2) PRV at an efficiency of 7.8%. Both efficiencies are greater than the wild-type OmpT (D97) and OmpT D97E variant (Asp 97 substituted by glutamic acid). It is also unexpected that OmpT D97H variant is able to cleave substrates having isoleucine (I), methionine (M), threonine (T), or asparagine (N) at the P'1 position. See Table 1 of the Specification (showing that no other OmpT variant is capable of cleaving PRI, PRM, PRT, or PRN).

(G) Reply: It is acknowledged that Example 13 teaches that the OmpT D97H variant cleaves PRE and PRD with an efficiency of less than 3%. However, the specification does not disclose by what percent efficiency the OmpT D97H variant cleaves PRE and PRD. Thus, one is not able to compare cleavage of PRE and PRD to other substrates, which range from 3.1-11% efficiency. Moreover, as explained in the rejection below, Ser, Thr, and Arg residues bind His (Wolf et al, 1995). Thus, cleavage of substrates having isoleucine (I), methionine (M), threonine (T), or asparagine (N) at the P'1 position is not unexpected.

Claims 49 and 51-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Wolf et al, 1995. As described in the prior action and above, the combination of Okuno et al, 2002a, Dekker et al, and Kramer et al renders obvious OmpT protease AAA24430.1 variants having a substitution at Asp^{97} with each of the naturally occurring amino acids, including **His,** and using said variants to cleave the fusion proteins of Okuno et al, 2002a (Tables 1&2) and the peptides of Dekker et al (Table 2), including those having a P1' of S, T, or R. It is noted that the fusion proteins of Okuno et al, 2002a have an Arg at P4 (Tables 1&2). As explained above, motivation to do so is provided by the desire to examine the P1' cleavage motif requirement of said variants by using the sequences of Okuno et al, and Dekker et al. The expectation of success is high, as the skilled artisan would have believed that,

more likely than not the OmpT protease variants having a substitution at Asp^{97} with **His** would cleave a substrate comprising a P1' that is S, T, or R. This expectation of success is based on the fact that (i) Kramer et al teaches that the residue at position 97 of OmpT protease determines substrate specificity in regards to position P1' (Fig 4), (ii) Wolf et al teaches that binding of histidine to HisJ is mediated by binding with S, T, and R residues (Table II&III; Fig 8), and (iii) substrate binding and cleavage is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 49 and 51-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Wolf et al, 1995.

Applicants' relevant arguments

As acknowledged by Applicants' (section 13.1) the prior rejection that is relevant to the rejection above is "(9) claims 50 and 65 over the combination of Okuno 2002a, Dekker, and Kramer, in view of Metzler". Applicants did not make any comments regarding this specific rejection because Claims 50 and 65 are cancelled. Claim 49 now encompasses the subject matter of prior Claims 50 and 65.

Regarding the above rejection of Claims 49 and 51-53 over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Wolf et al, 1995, Applicants provided the same arguments, as set forth above regarding the rejection of Claims 49 and 51-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Okuno et al, 2002a, Dekker et al, 2001, and Kramer et al, 2001 in view of Metzler, 2001. Said arguments are not persuasive for the reasons set forth above.

Claims 54-56 59-61, 67, and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, and Kramer et al, 2001 in view of

Yamamoto et al, 1996 and Metzler, 2001. As described in the prior action, the combination of Stumpe et al, Suzuki et al, and Kramer et al teaches using OmpT protease AAA24430.1 to cleave the protamine component Clupein YII between ^{23}Arg-Arg-Arg-Ala-Arg↓Arg^{28}, wherein positions P5-P1 are as set forth by SEQ ID NO: 11. Said combination does not teach cleaving a fusion protein comprising the target protein motilin (FVPIFTYGELQRMQEKERNKGQ) using an OmpT variant having a substitution of **Asp^{97}Met** substitution. As explained in the prior action, Yamamoto et al teaches making a fusion protein comprising the target protein motilin (FVPIFTYGELQRMQEKERNKGQ) in E. coli host cells (Col 4-Formula 1a (A-B-C); Col 7, ¶2; Example 1) and cleaving said fusion protein, at a linker motif comprising Lys↓Arg, using OmpT protease (Col 6, parg5). Yamamoto et al does not teach cleaving a fusion protein wherein the P1 residue is the penultimate Arg of Clupein YII and the P1' residue is the N-terminal Phe of motilin, i.e. RRRAR↓FVPIFTYGELQRMQEKERNKGQ.

Dekker et al teaches that OmpT protease has 5-50% cleavage activity at the motif Arg↓Phe-Val (Table 2). The art teaches that amino acids can be classified based on charge, hydrophobicity, and size (Metzler, 2001). Thus, Met is considered to be a neutral/hydrophobic amino acid. It would have been obvious to a person of ordinary skill in the art to make the OmpT protease AAA24430.1 variant having a substitution at Asp^{97} with neutral/hydrophobic amino acids, including Ala, Val, Ile, Phe, Tyr, and Trp, **Met** and Leu, and use said variants to cleave a fusion protein wherein the P1 residue is the penultimate Arg of Clupein YII and the P1' residue is the N-terminal Phe of motilin, i.e. RRRAR↓FVPIFTYGELQRMQEKERNKGQ. Motivation to do so is provide by the desire to isolate motilin without additional amino acids. The expectation of success is high, as the skilled artisan would have believed that, more likely than not the OmpT protease variant having a substitution at Asp^{97} with **Met** would cleave a substrate comprising a P1' of **F**. This expectation of success is based on the fact

that (i) Kramer et al teaches that the residue at position 97 of OmpT protease determines substrate specificity at the position P1' (Fig 4), (ii) neutral/hydrophobic amino acids are more likely to associate with each other (Met with Phe), and (iii) substrate binding and cleavage is also coordinated by residues Glu^{27}, Asp^{83}, Asp^{85}, Asp^{208}, Asp^{210}, and His^{212} of OmpT protease (Kramer et al; Fig 4). Therefore, Claims 54-56 59-61, 67, and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Stumpe et al, 1998, Suzuki et al, 1972, and Kramer et al, 2001 in view of Yamamoto et al, 1996 and Metzler, 2001.

Applicants' relevant arguments

Regarding Claims 54-56 59-61, 67, and 68, as amended, Applicants present the following arguments (sections 13.3&13.4), which are relevant to the rejection above. These arguments are not found to be persuasive for the reasons in the reply.

(A) Applicants reiterate their arguments regarding Claim 49, as set forth above.

(A) Reply: These arguments are not found to be persuasive for the reasons set forth above.

Allowable Subject Matter

No claims are allowable.

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Regarding filing an Appeal, Applicants are referred to the Official Gazette Notice published July 12, 2005 describing the Pre-Appeal Brief Review Program.

Final Comments

To insure that each document is properly filed in the electronic file wrapper, it is requested that each of amendments to the specification, amendments to the claims, Applicants' remarks, requests for extension of time, and any other distinct papers be submitted on separate pages. It is also requested that the serial number of the application be referenced on every page of the response.

It is also requested that Applicants identify support, within the original application, for any amendments to the claims and specification.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SHERIDAN SWOPE whose telephone number is 571-272-0943. The examiner can normally be reached on 11a-7:30p7 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi, can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published application may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on the access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SHERIDAN SWOPE/
Primary Examiner, Art Unit 1652